

# A theory for the membrane potential of cells.

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## ABSTRACT

We give an explicit formula for the membrane potential of cells in terms of the intracellular and extracellular ionic concentrations, and derive equations for the ionic currents that flow through channels, exchangers and electrogenic pumps based on simple energy considerations and conservation laws. We demonstrate that the work done by the pump is equal to the potential energy of the cell plus the energy loss due to the downhill ionic fluxes through the channels and the exchanger. Our equations predict osmotic pressure variations. The theory is illustrated in a simple model of spontaneously active cells in the cardiac pacemaker. The simulated action potential and the five currents in the model are in excellent agreement with experiments. The model predicts the experimental observed intracellular ionic concentration of potassium, calcium and sodium. We do not see any drift of the values for the concentrations in a long time simulation, instead we can obtain the same asymptotic values starting with equal intracellular and extracellular ionic concentrations.

## I. INTRODUCTION

Ionic models of the cellular membrane potential are typically guided by extensive data sets from voltage clamp experiments. The purpose of this paper is not to introduce a new ionic model incorporating the many experimentally observed currents (Boyett, 1996), or to critically review other models, but merely to give an alternative formulation based on simple principles from classical physics. Instead of trying to reproduce observed voltage clamp data and tail currents of activation, deactivation and inactivation, we demonstrate that simple energy considerations and conservation laws can be useful when formulating a model.

We derive equations for ionic currents flowing through channels, exchangers and electrogenic pumps. These are based on the Boltzmann distribution law (Boltzmann, 1868), which states that a particle in thermal equilibrium spends less time in states of higher energy than in states of lower energy, the Markov assumption (Markov, 1906) which says that the transition probabilities to the next state of a stochastic system is only dependent on its present state, and the principle of detailed balance (Onsager, 1931) which says that the microscopic laws of physics are invariant with respect to the reversal of time. Our equations were inspired by Ehrenstein and Lecar's model of channel gating (1977), Mullins' model of the  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  exchanger (1977), and Chapman's model of the  $\text{Na}^+$ ,  $\text{K}^+$  pump (1978).

Since the intracellular ionic concentrations are treated as dynamical variables, we show that the standard differential equation for the membrane potential can be replaced with a new algebraic equation. We demonstrate that the pump generates a transmembrane voltage gradient, a transmembrane pressure gradient, transmembrane concentration gradients, and downhill ionic fluxes through the exchanger and the channels.

The theory is illustrated with a simple model of spontaneously active cells in the rabbit sinoatrial node. The observable parameters in the model are based on the experiments of Shibasaki (1987), Hagiwara *et al.* (1988), Muramatsu *et al.* (1996) and Sakai *et al.* (1996). The non-observable parameters in the model are determined numerically, in the same way as in an earlier study (Endresen, 1997a), by comparing the action potentials generated by the model with the action potentials recorded by Baruscotti *et al.* (1996).

## II. DERIVATION OF THE EQUATIONS

### A. Nernst Equation

There are two basic principles behind the average motion of particles. The first is diffusion which is general; the second applies only to charged particles such as ions in solutions. Simple diffusion is described by the empirical law of Fick:

$$\vec{\phi} = -ukT\nabla[S] , \quad (1)$$

where  $\phi$  is the ionic flux,  $[S]$  the concentration of ions and  $u$  the ratio of the velocity to the force acting on a particle, known as the mobility. The empirical law of Ohm describes the net motion of charged particles in an electric field:

$$\vec{\phi} = -zeu[S]\nabla v , \quad (2)$$

where  $z$  is the valence,  $e$  the elementary charge and  $v$  the potential. Since we assume that the temperature is constant, we can neglect the thermal flux given by Fourier's empirical law. The fact that the mobility in Fick's law must be identical to the mobility in Ohm's law was first noticed by Einstein (1905). If we combine equations (1) and (2), the total flux of ions due to diffusion and electric forces becomes:

$$\vec{\phi} = -ukT \exp\left(-\frac{zev}{kT}\right) \nabla \left[ [S] \exp\left(\frac{zev}{kT}\right) \right] . \quad (3)$$

The equilibrium potential where the flux is zero can be found by integrating equation (3) from the intracellular (i) to the extracellular (e) side of the membrane:

$$v_S = v_i - v_e = \frac{kT}{ze} \ln \frac{[S]_e}{[S]_i} , \quad (4)$$

where  $[S]_i$  and  $[S]_e$  refer to the intracellular and extracellular concentrations. This equation, first stated by Nernst (1888) is based only on the empirical laws of Ohm and Fick and the relation of Einstein.

The same formula can be derived in a more general way using the Boltzmann factor (Boltzmann, 1868). The relative probability at equilibrium that an ion is at the intracellular or extracellular side of a cell membrane is

$$\frac{p_e}{p_i} = \frac{[S]_e}{[S]_i} = \exp\left(-\frac{\Delta w}{kT}\right) , \quad (5)$$

where  $\Delta w$  is the energy difference between these intra- and extracellular states,  $T$  is the absolute temperature and  $k$  is Boltzmann's constant. If we consider ions of valence  $z$  the energy difference between the intracellular and extracellular side can be written:

$$\Delta w = ze(v_e - v_i) . \quad (6)$$

This together with equation (5) yields equation (4). The equilibrium potentials for the predominant cellular cations are then:

$$v_K = \frac{kT}{e} \ln \frac{[K]_e}{[K]_i} , \quad (7)$$

$$v_{Ca} = \frac{kT}{2e} \ln \frac{[Ca]_e}{[Ca]_i} , \quad (8)$$

$$v_{Na} = \frac{kT}{e} \ln \frac{[Na]_e}{[Na]_i} . \quad (9)$$

## B. Ionic Channels

We assume that ionic channels are either completely open or completely closed and randomly fluctuate between these states. Each channel is here assumed to be a simple Markov process (Markov, 1906), described by first order kinetics::

$$C \xrightleftharpoons[\beta]{\alpha} O , \quad (10)$$

where the rate constants  $\alpha$  and  $\beta$  are functions of transmembrane voltage and control the transitions between the closed ( $C$ ) and the open ( $O$ ) states of the channel. The rate for a closed channel to open is  $\alpha$ , and  $\beta$  is the rate for an open channel to close. Let  $x$  denote the average fraction of channels that are open, or, equivalently, the probability

that a given channel will be open. We may say that the ionic flux through an ensemble of channels is regulated by a sliding door whose position is  $x$ . This yields:

$$\frac{dx}{dt} = \alpha(1 - x) - \beta x \equiv \frac{x_\infty - x}{\tau}, \quad (11)$$

where

$$x_\infty = \frac{\alpha}{\alpha + \beta} \quad (12)$$

$$\tau = \frac{1}{\alpha + \beta}. \quad (13)$$

Here  $x_\infty$  denotes the steady state fraction of open channels and  $\tau$  the relaxation time. Let us assume that the energy difference between the closed and open positions is given by

$$\Delta w = w_{\text{open}} - w_{\text{closed}} \equiv q(v_x - v), \quad (14)$$

where  $q$  is an gating charge, usually  $q \approx 4e$ , the term  $qv_x$  is due to the difference in mechanical conformational energy between the two states and  $qv$  represents the change in electrical potential energy due to the redistribution of charge during the transition. At equilibrium,  $dx/dt = 0$ , and the ratio of the probabilities for a single channel to be in the open state or the closed state is,

$$\frac{x_\infty}{1 - x_\infty} = \frac{\alpha}{\beta}. \quad (15)$$

This relation is known as the principle of detailed balance (Onsager, 1931). The same ratio is given by the Boltzmann distribution (Boltzmann, 1868),

$$\frac{x_\infty}{1 - x_\infty} = \exp\left(-\frac{\Delta w}{kT}\right). \quad (16)$$

Thus, from equations (14), (15) and (16):

$$x_\infty = \left(1 + \exp\left[\frac{(v_x - v)}{\frac{kT}{4e}}\right]\right)^{-1}, \quad (17)$$

and

$$\frac{\alpha}{\beta} = \frac{\exp\left[-\frac{(v_x - v)}{\frac{kT}{2e}}\right]}{\exp\left[+\frac{(v_x - v)}{\frac{kT}{2e}}\right]}. \quad (18)$$

Therefore, making the *ad hoc* assumption that the product of forward and backward reaction rates is constant, the symmetric choice for  $\alpha$  and  $\beta$  is:

$$\alpha = \lambda \exp\left[-\frac{(v_x - v)}{\frac{kT}{2e}}\right] \quad (19)$$

$$\beta = \lambda \exp\left[+\frac{(v_x - v)}{\frac{kT}{2e}}\right], \quad (20)$$

where  $\lambda$  is a constant. Taking  $\lambda$  to be constant gives the maximum relaxation time at the voltage where  $x_\infty = 1/2$ . The relaxation time is then:

$$\tau = \frac{1}{\alpha + \beta} = \left(2\lambda \cosh\left[\frac{q(V_1 - V)}{2kT}\right]\right)^{-1}. \quad (21)$$

### 1. Potassium channels

If the flux of ions is driven by Ohm's law and regulated by the fraction of open channels  $x$ , the membrane current through potassium channels becomes:

$$\begin{aligned} i_K &= g_K x (v - v_K) \\ \frac{dx}{dt} &= \tau_K^{-1} \cosh \left\{ \frac{v - v_x}{\frac{kT}{2e}} \right\} \left[ \frac{1}{2} \left( 1 + \tanh \left\{ \frac{v - v_x}{\frac{kT}{2e}} \right\} \right) - x \right], \end{aligned} \quad (22)$$

where  $\tau_K = 1/2\lambda$  is the maximum value of the relaxation time,  $g_K$  is the conductance,  $v_K$  is given by equation (7), where we have used the mathematical identity:

$$\frac{1}{2} [1 + \tanh(\phi)] = \frac{1}{1 + \exp(-2\phi)}. \quad (23)$$

### 2. Calcium and sodium channels

The calcium and sodium channels also have an inactivation mechanism in addition to the above activation mechanism. We can view these mechanisms as two independent Markov processes, or equivalently two independent sliding doors, which are both affected by voltage. An ion can only go through if both sliding doors are at least slightly open. The activation mechanism is very fast, so we use the steady state fraction of open channels (equation (18)) for this. The maximum time constant of inactivation for calcium and sodium channels are of the same order of magnitude as the maximum time constant of the activation of the potassium channel, thus:

$$\begin{aligned} i_{Ca} &= g_{Ca} f (v - v_{Ca}) \frac{1}{2} \left( 1 + \tanh \left\{ \frac{v - v_d}{\frac{kT}{2e}} \right\} \right) \\ \frac{df}{dt} &= \tau_{Ca}^{-1} \cosh \left\{ \frac{v - v_f}{\frac{kT}{2e}} \right\} \left[ \frac{1}{2} \left( 1 - \tanh \left\{ \frac{v - v_f}{\frac{kT}{2e}} \right\} \right) - f \right], \end{aligned} \quad (24)$$

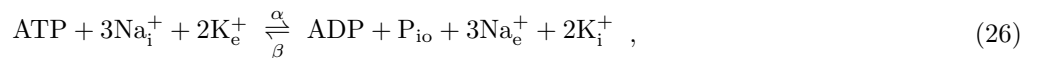
and,

$$\begin{aligned} i_{Na} &= g_{Na} h (v - v_{Na}) \frac{1}{2} \left( 1 + \tanh \left\{ \frac{v - v_m}{\frac{kT}{2e}} \right\} \right) \\ \frac{dh}{dt} &= \tau_{Na}^{-1} \cosh \left\{ \frac{v - v_h}{\frac{kT}{2e}} \right\} \left[ \frac{1}{2} \left( 1 - \tanh \left\{ \frac{v - v_h}{\frac{kT}{2e}} \right\} \right) - h \right], \end{aligned} \quad (25)$$

where  $g_{Ca}$  and  $g_{Na}$  are the conductances of the calcium and sodium currents respectively,  $v_{Ca}$  and  $v_{Na}$  are given by equations (8) and (9),  $v_d$  and  $v_m$  are the half-activation potentials, and  $v_f$  and  $v_h$  are the half-inactivation potentials.

### C. $\text{Na}^+, \text{K}^+$ Pump

The Na,K-ATPase is found in the plasma membrane of virtually all animal cells and is responsible for active transport of sodium and potassium. Low sodium concentration and high potassium concentration in the cytosol are essential for basic cellular functions such as excitability, secondary active transport, and volume regulation. In our model, the  $\text{Na}^+, \text{K}^+$  pump is the only energy source. We shall assume that the following equation is a complete macroscopic description of the pump reaction (Chapman, 1978):



where ATP, ADP and  $\text{P}_{io}$  are adenosine triphosphate, adenosine diphosphate and inorganic phosphate, while  $\alpha$  and  $\beta$  are the rates of the forward and backward reactions. The energy involved in the movement of 3  $\text{Na}^+$  and 2  $\text{K}^+$  ions against their electrochemical gradients is:

$$\Delta w_{\text{Na}} = -3e(v - v_{\text{Na}}) \quad (27)$$

$$\Delta w_{\text{K}} = +2e(v - v_{\text{K}}) , \quad (28)$$

where  $v_{\text{K}}$  and  $v_{\text{Na}}$  are given by equations (7) and (9). This result is independent of the detailed interaction between ions, molecules and the ATPase enzyme. Therefore, the total work done in reaction (26) is:

$$\begin{aligned} \Delta w &= \Delta w_{\text{ATP}} + \Delta w_{\text{Na}} + \Delta w_{\text{K}} \\ &= \Delta w_{\text{ATP}} + e(3v_{\text{Na}} - 2v_{\text{K}} - v) , \end{aligned} \quad (29)$$

where  $\Delta w_{\text{ATP}}$  is the energy associated with the breakdown of ATP. The ratio of  $\alpha$  to  $\beta$  is again given by equations (15) and (16). The energy available from ATP breakdown is much larger than the energy required to translocate the potassium and sodium ions at small negative membrane potentials. In practice, such a pump or motorized swing door will soon reach saturation. We therefore choose the rate of the forward reaction to be constant, resembling the maximum possible speed of the swing door in that direction, and obtain:

$$\alpha = \lambda \quad (30)$$

$$\beta = \lambda \exp \left[ \frac{\Delta w_{\text{ATP}} + e(3v_{\text{Na}} - 2v_{\text{K}} - v)}{kT} \right] , \quad (31)$$

where  $\lambda$  is a constant. The net pump current for a cell with  $M$  pumps can then be written:

$$i_{\text{NaK}} = eM(\alpha - \beta) = k_{\text{NaK}} \left( 1 - \exp \left\{ \frac{-v - 2v_{\text{K}} + 3v_{\text{Na}} + v_{\text{ATP}}}{\frac{kT}{e}} \right\} \right) , \quad (32)$$

where  $k_{\text{NaK}} = e\lambda M$ , and  $v_{\text{ATP}} = \Delta w_{\text{ATP}}/e$ .

#### D. $\text{Na}^+, \text{Ca}^{2+}$ Exchanger

To maintain a steady state for the intracellular calcium concentration in for example heart cells, the amount of calcium that enters the cell via ionic channels must be extruded. The  $\text{Na}^+, \text{Ca}^{2+}$  exchanger is the major mechanism responsible for achieving a balance between calcium entry and extrusion in oscillating cells. We assume that the rates of the exchange reaction (Mullins, 1977) given by:

$$3\text{Na}_e^+ + \text{Ca}_i^{2+} \xrightleftharpoons[\beta]{\alpha} 3\text{Na}_i^+ + \text{Ca}_e^{2+} , \quad (33)$$

are governed largely by the electrochemical gradients for sodium and calcium, together with the membrane potential. In other words, the energy produced when 3 extracellular sodium ions take the elevator down into the cytosol is used to elevate one calcium ion up from the cytosol into the extracellular space:

$$\Delta w_{\text{Na}} = +3e(v - v_{\text{Na}}) \quad (34)$$

$$\Delta w_{\text{Ca}} = -2e(v - v_{\text{Ca}}) , \quad (35)$$

where  $v_{\text{Ca}}$  and  $v_{\text{Na}}$  are given by equations (8) and (9). The total work done in reaction (33) is:

$$\Delta w = \Delta w_{\text{Na}} + \Delta w_{\text{Ca}} = e(v - 3v_{\text{Na}} + 2v_{\text{Ca}}) . \quad (36)$$

The ratio of  $\alpha$  to  $\beta$  in equation (33) is given by equations (15) and (16). Since  $\Delta w = e(v - 3v_{\text{Na}} + 2v_{\text{Ca}})$  in a cell usually oscillates around zero, the most natural choice of  $\alpha$  and  $\beta$  is:

$$\alpha = \lambda \exp \left[ -\frac{e(v - 3v_{\text{Na}} + 2v_{\text{Ca}})}{2kT} \right] \quad (37)$$

$$\beta = \lambda \exp \left[ +\frac{e(v - 3v_{\text{Na}} + 2v_{\text{Ca}})}{2kT} \right] , \quad (38)$$

where again we make the *ad hoc* assumption that  $\lambda$  is a constant. For a cell with  $N$  exchangers the net current is then:

$$i_{\text{NaCa}} = -eN(\alpha - \beta) = k_{\text{NaCa}} \sinh \left\{ \frac{v - 3v_{\text{Na}} + 2v_{\text{Ca}}}{\frac{2kT}{e}} \right\} , \quad (39)$$

where  $k_{\text{NaCa}} = 2e\lambda N$ .

## E. Membrane Voltage

We assume that the electrical activity of a cell is described by the five currents discussed above, and that all the other currents (Boyett, 1996) are of minor importance. The standard differential equation for the voltage, and the conservation laws for intracellular ionic concentrations are then:

$$\frac{dv}{dt} = -\frac{1}{C} (i_K + i_{Ca} + i_{Na} + i_{NaCa} + i_{NaK}) , \quad (40)$$

$$\frac{d}{dt} [K]_i = \frac{2i_{NaK} - i_K}{FV} , \quad (41)$$

$$\frac{d}{dt} [Ca]_i = \frac{2i_{NaCa} - i_{Ca}}{2FV} , \quad (42)$$

$$\frac{d}{dt} [Na]_i = \frac{-i_{Na} - 3i_{NaK} - 3i_{NaCa}}{FV} , \quad (43)$$

where  $C$  is cell capacitance,  $F$  is Faraday's constant and we assume that the cell volume  $V$  is constant. Solving equation (41) for  $i_K$ , equation (42) for  $i_{Ca}$ , and equation (43) for  $i_{Na}$ , we obtain:

$$i_K = -FV \frac{d}{dt} [K]_i + 2i_{NaK} , \quad (44)$$

$$i_{Ca} = -2FV \frac{d}{dt} [Ca]_i + 2i_{NaCa} , \quad (45)$$

$$i_{Na} = -FV \frac{d}{dt} [Na]_i - 3i_{NaK} - 3i_{NaCa} . \quad (46)$$

Inserting this into equation (40) we obtain:

$$\frac{dv}{dt} = \frac{FV}{C} \frac{d}{dt} ([K]_i + 2[Ca]_i + [Na]_i) , \quad (47)$$

since all of the currents cancel. This equation can also be written as:

$$\frac{d}{dt} \left( v - \frac{FV}{C} \{ [K]_i + 2[Ca]_i + [Na]_i \} \right) = 0 . \quad (48)$$

Integrating gives:

$$v - \frac{FV}{C} ([K]_i + 2[Ca]_i + [Na]_i) = c , \quad (49)$$

where the integration constant  $c$  has to be determined. Given that the voltage across a capacitor is zero when the net charge difference is zero, choosing the integration constant as:

$$c = -\frac{FV}{C} \{ [K]_e + 2[Ca]_e + [Na]_e \} , \quad (50)$$

gives:

$$v = \frac{FV}{C} \{ ([K]_i - [K]_e) + 2([Ca]_i - [Ca]_e) + ([Na]_i - [Na]_e) \} , \quad (51)$$

so that the voltage is zero when there is no charge gradient. Equation (51) is simply the relation between the electric potential and charge of a capacitor, which is in fact the origin of equation (40). Thus it is completely general and independent of the number of membrane currents in a model. The meaning of equation (51) is:

*The voltage across the membrane of a cell is caused by, and is directly proportional to, the surplus of charge inside the cell,*

but it is only valid if we assume that the intracellular concentration of anions is equal to the extracellular concentration of anions. Since the cations are balanced by the anions in any solution, the intracellular/extracellular concentration of anions must be close to the intracellular/extracellular concentration of cations.

Since equation (51) is the explicit integral of equation (40), it should be used instead of equation (40) (or the equivalent of equation (40)) in any model. The differential equation (40) is needed only in models where the intracellular ionic concentrations are not tracked individually (for example, the Hodgkin–Huxley equations (1952)).

There is a significant difference between equations (40) and (51). Here is an analogy: There are two different ways to calculate how many ions are inside a cell. The first method counts every ion entering or leaving (equation (40)), while the second method simultaneously counts all the ions inside the cell, perhaps by means of an ion detector (equation (51)). Both methods will correctly give the *variation* in the number of ions in the cell. However, the observer of ions entering and leaving will only observe *variations* in the number of ions. If he wants to know the actual number of ions in the cell, he must make an initial guess of the number of ions already inside. Because his guess may differ significantly from the actual ion number (as measured by the ion detector) indicates, the results from the two methods may be contradictory.

## F. Osmotic Pressure

In this section we demonstrate that variations in the osmotic pressure are a natural consequence of our model. In fact, van't Hoff's equation for the osmotic pressure can be derived by tracking the flow of energy under the assumption of constant temperature and volume.

From elementary physics the work  $W$  done by a force  $\mathcal{F}$  to move an object from  $\mathcal{X} = \mathcal{A}$  to  $\mathcal{X} = \mathcal{B}$  is given by:

$$W = \int_{\mathcal{A}}^{\mathcal{B}} \mathcal{F} d\mathcal{X} = \mathcal{F}(\mathcal{B} - \mathcal{A}) = \int_0^t \mathcal{F} \frac{d\mathcal{X}}{dt} dt = \int_0^t \mathcal{F} \mathcal{V} dt, \quad (52)$$

where  $\mathcal{V} = \frac{d\mathcal{X}}{dt}$  is the velocity of the object, and  $\mathcal{X}(0) = \mathcal{A}$ ,  $\mathcal{X}(t) = \mathcal{B}$ . When ions move across the membrane of a cell the transmembrane voltage  $v$  is like a force and the current  $i$  is like a velocity, thus:

$$W = \int_0^t v i dt. \quad (53)$$

If we take into account the reversal potentials in our model this can be written:

$$\begin{aligned} W = & \int_0^t i_K(v - v_K) + i_{Ca}(v - v_{Ca}) + i_{Na}(v - v_{Na}) \\ & + i_{NaCa}(v - 3v_{Na} + 2v_{Ca}) + i_{NaK}(v + 2v_K - 3v_{Na}) dt. \end{aligned} \quad (54)$$

Using equations (40), (44), (45) and (46) the currents cancel and we obtain:

$$W = -C \int_0^v v dv + FV \int_{[K]_e}^{[K]_i} v_K d([K]_i) + 2FV \int_{[Ca]_e}^{[Ca]_i} v_{Ca} d([Ca]_i) + FV \int_{[Na]_e}^{[Na]_i} v_{Na} d([Na]_i) \quad (55)$$

When solving these integrals it is important to remember that the reversal potentials  $v_K$ ,  $v_{Ca}$  and  $v_{Na}$  given by equations (7), (8) and (9), are dependent on the integration variables  $[K]_i$ ,  $[Ca]_i$  and  $[Na]_i$ . Using:

$$\int \ln(\phi) d\phi = \phi \ln(\phi) - \phi, \quad (56)$$

the integral becomes:

$$\begin{aligned} W = & -\frac{1}{2} C v^2 - RTV \left\{ [K]_i \ln\left(\frac{[K]_i}{[K]_e}\right) + [Ca]_i \ln\left(\frac{[Ca]_i}{[Ca]_e}\right) + [Na]_i \ln\left(\frac{[Na]_i}{[Na]_e}\right) \right\} \\ & - RTV \{ [K]_e - [K]_i + [Na]_e - [Na]_i + [Ca]_e - [Ca]_i \}. \end{aligned} \quad (57)$$

This is the total work associated with the currents. The pump work and work due to the exchanger and channel currents have opposite signs since the pump moves ions against their electrochemical gradients, while energy is lost by the downhill ionic fluxes through the exchanger and the channels. Since energy cannot be created or destroyed, we define the potential energy  $P$  of the cell so that the total energy  $E$  is zero (this is the most common definition of potential energy):

$$E = P + W = 0 , \quad (58)$$

thus,

$$P = \frac{1}{2}Cv^2 + RTV \left\{ [K]_i \ln\left(\frac{[K]_i}{[K]_e}\right) + [Ca]_i \ln\left(\frac{[Ca]_i}{[Ca]_e}\right) + [Na]_i \ln\left(\frac{[Na]_i}{[Na]_e}\right) \right\} \\ + RTV \{ [K]_e - [K]_i + [Na]_e - [Na]_i + [Ca]_e - [Ca]_i \} . \quad (59)$$

The first term is the electrical potential energy of a capacitor, the second term is the potential energy associated with the concentration gradients, and the third term is the potential energy associated with an osmotic pressure gradient (caused by the concentration gradients). Rewriting equation (58):

$$\int_0^t i_{NaK}(v + 2v_K - 3v_{Na})dt = \quad (60)$$

$$- \frac{1}{2}Cv^2 \quad (61)$$

$$- RTV \left\{ [K]_i \ln\left(\frac{[K]_i}{[K]_e}\right) + [Ca]_i \ln\left(\frac{[Ca]_i}{[Ca]_e}\right) + [Na]_i \ln\left(\frac{[Na]_i}{[Na]_e}\right) \right\} \quad (62)$$

$$- RTV \{ [K]_e - [K]_i + [Na]_e - [Na]_i + [Ca]_e - [Ca]_i \} \quad (63)$$

$$- \int_0^t i_K(v - v_K) + i_{Ca}(v - v_{Ca}) + i_{Na}(v - v_{Na}) + i_{NaCa}(v - 3v_{Na} + 2v_{Ca})dt , \quad (64)$$

we see that the pump (equation (60)) produces:

1. a transmembrane voltage gradient (equation (61)),
2. a transmembrane concentration gradient (equation (62)),
3. a transmembrane pressure gradient (equation (63)), and
4. downhill fluxes through the exchanger and the channels (equation (64)).

In 1886 van't Hoff noticed that the behaviour of solutes in dilute solutions resembles the behaviour of a perfect gas:

The pressure which a gas exerts when a given number of molecules are distributed in a given volume is equally great as the osmotic pressure, which under the same conditions would be produced by most solutes when they are dissolved in an arbitrary solvent.

Therefore we can use the ideal gas law  $p = [S]RT$  to calculate the osmotic pressure across the cell membrane:

$$\pi = RT \{ [K]_i - [K]_e + [Na]_i - [Na]_e + [Ca]_i - [Ca]_e \} . \quad (65)$$

This is the van't Hoff equation for the osmotic pressure ( $\pi$ ) across a solute impermeable barrier separating two ideal dilute solutions. This is nothing but equation (63) divided by the cell volume  $V$ .

Since we did not have the osmotic pressure in mind when we made the theory, our theory is a perfect example of one of Richard P. Feynman's astute observations:

When you have put a lot of ideas together to make an elaborate theory, you want to make sure, when explaining what it fits, that those things it fits are not just the things that gave you the idea of the theory; but that the finished theory makes something else come out right, in addition.

Our theory indeed has “something else [that] comes out right” – the osmotic pressure variations.



### III. MODEL PARAMETERS

A mathematical model of the membrane potential has been derived; equations (7), (8) and (9) represent the equilibrium potentials, equations (22), (24) and (25) the ionic currents, equations (39) and (32) the exchanger and the pump currents, equations (41), (42) and (43) the ionic concentrations, equation (51) the membrane voltage, and finally, equation (65) the osmotic pressure across the cell membrane. The model has 6 dimensions with the variables  $x$ ,  $f$ ,  $h$ ,  $[K]_i$ ,  $[Ca]_i$  and  $[Na]_i$ .

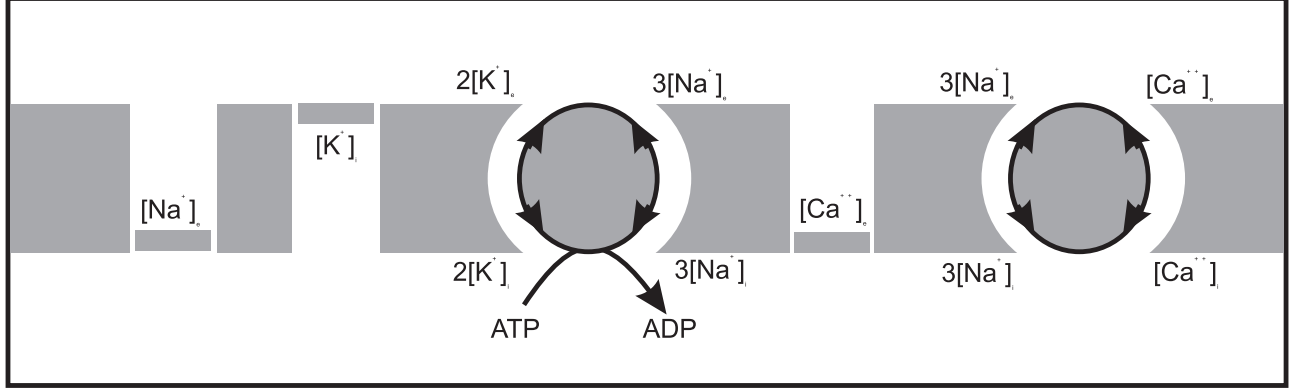


FIG. 1. Schematic diagram of the simplified cell model. The three ionic currents of potassium, calcium and sodium, the exchanger and the pump. The “moveable” gating doors of the ionic channels illustrate that the concentration gradient of potassium is the opposite to the concentration gradients of calcium and sodium.

Fig. 1 shows a schematic diagram of the model, with the ionic channels of potassium, calcium and sodium, the  $Na^+$ ,  $Ca^{2+}$  exchanger and the  $Na^+$ ,  $K^+$  pump. The “moveable” gating doors of the ionic channels illustrate that the concentration gradient of potassium is the opposite of the concentration gradients of calcium and sodium.

On the basis of distinct biophysical and pharmacological properties, cardiac calcium currents have been classified into a long lasting type ( $i_{Ca,L}$ ) and a transient type ( $i_{Ca,T}$ ), while cardiac delayed rectifier potassium currents have been classified into a rapid type ( $i_{Kr}$ ) and a slow type ( $i_{Ks}$ ). In our model we assume that  $i_{Ca,T}$  and  $i_{Ks}$  are of minor importance; i.e. when we talk about  $i_{Ca}$  we mean  $i_{Ca,L}$ , and when we talk about  $i_K$  we mean  $i_{Kr}$ .

First, we want to justify the presence of the term  $kT/2e$  in equations (22), (24) and (25). This corresponds to a slope factor for the activation and inactivation curves of  $kT/4e \approx 6.68\text{mV}$  at  $37^\circ\text{C}$ . The observed slope factors are  $7.4\text{mV}$  for activation of  $i_K$  (Shibasaki, 1987),  $6.6\text{mV}$  for activation of  $i_{Ca}$  (Hagiwara *et al.*, 1988),  $6.0\text{mV}$  for inactivation of  $i_{Ca}$  (Hagiwara *et al.*, 1988),  $6.0\text{mV}$  for activation of  $i_{Na}$  (Muramatsu *et al.*, 1996), and, finally,  $6.4\text{mV}$  for inactivation of  $i_{Na}$  (Muramatsu *et al.*, 1996). Hence, we see that  $kT/4e$ , corresponding to a gating charge of  $q \approx 4e$ , is an excellent approximation. Now, we would like to distinguish between the fundamental physical constants (table I), the experimentally observed constants (table II), the adjustable parameters (table III) and the initial conditions (table IV) in the model.

TABLE I. Fundamental Physical Constants

Name	Value	Unit
$k$	$1.38065812 \cdot 10^{-20}$	mJ/K
$e$	$1.6021773349 \cdot 10^{-19}$	C
$F$	96485.30929	C/mol
$T$	310.15	K
$R = kF/e$	8314.511935	J/kmol K

The half-activation and inactivation potentials in the model ( $v_x$ ,  $v_d$ ,  $v_f$ ,  $v_m$  and  $v_h$ ) are based on the experiments of Shibasaki (1987), Hagiwara *et al.* (1988) and Muramatsu *et al.* (1996), and we use a value of  $v_{ATP}$  that gives a reversal potential for the sodium pump in good agreement with the experiments of Sakai *et al.* (1996). The maximum time constants in these experiments were 203ms for activation of  $i_K$  (Shibasaki, 1987), 225ms for inactivation of  $i_{Ca}$  (Hagiwara *et al.*, 1988) and 174ms for inactivation of  $i_{Na}$  (Muramatsu *et al.*

*al.*, 1996). In the model, however, we combine these and use a maximum time constant of 200 ms for both  $\tau_K$ ,  $\tau_{Ca}$  and  $\tau_{Na}$ . Finally, we use typical values for cell volume, cell capacitance, and extracellular ionic concentrations.

TABLE II. Observed Constants

Name	Value	Unit
$[K]_e$	5.4	mM
$[Ca]_e$	2	mM
$[Na]_e$	140	mM
$V$	10	$10^3 \mu m^3$
$C$	47	pF
$v_x$	-25.1	mV
$v_d$	-6.6	mV
$v_f$	-25.0	mV
$v_m$	-41.4	mV
$v_h$	-91.0	mV
$v_{ATP}$	-450	mV
$\tau$	200	ms

The density of ionic channels, exchangers and pumps (i.e.  $g_{Ca}$ ,  $g_{Na}$ ,  $g_K$ ,  $k_{NaK}$  and  $k_{NaCa}$ ) can vary significantly from cell to cell. In order to reproduce the action potentials recorded in a spontaneously beating adult rabbit sinoatrial node cell with normal Tyrode solution as external super fusing solution (Fig. 7 A. in Baruscotti *et al.* (1996)), we fit the adjustable parameters (table III) and the initial conditions (table IV) numerically. More details are given in (Endresen, 1997a). Many combinations of  $g_{Ca}$ ,  $g_{Na}$ ,  $g_K$ ,  $k_{NaK}$  and  $k_{NaCa}$  resulted in good approximations to the experimentally recorded waveform, from which we conclude that different cells can produce the same action potential although they have a different mixture of ionic channels, exchangers and pumps. The final set of parameters presented in table III are based on the choice  $k_{NaK} = 12.2$  pA.

TABLE III. Adjustable Parameters

Name	Value	Unit
$g_{Ca}$	9.29045	nS
$g_{Na}$	253.94203	nS
$g_K$	0.70302	nS
$k_{NaCa}$	8181.31568	pA
$k_{NaK}$	12.20000	pA

TABLE IV. Initial Conditions

Name	Value	Unit
$x_0$	0	-
$f_0$	1	-
$h_0$	0	-
$[K]_{i_0}$	130.880955	mM
$[Ca]_{i_0}$	0.000790	mM
$[Na]_{i_0}$	18.514880	mM

#### IV. SIMULATION RESULTS

The six differential equations in the model were solved numerically using a fifth-order Runge-Kutta method with variable steplength, more details are given in (Endresen, 1997b). Fig. 2 shows the five membrane currents and Fig. 3 shows the recorded and simulated action potential together with the intracellular ionic concentrations. These computations used the initial conditions stated in table IV.

Cells must generate their membrane potential by actively transporting ions against their concentration gradients. To examine this process in our model, we ran a simulation starting with equal intracellular and extracellular ionic concentrations:

$$\begin{aligned} [K]_i &= 5.4 \text{ mM} , \\ [Ca]_i &= 2 \text{ mM} , \\ [Na]_i &= 140 \text{ mM} . \end{aligned} \tag{66}$$

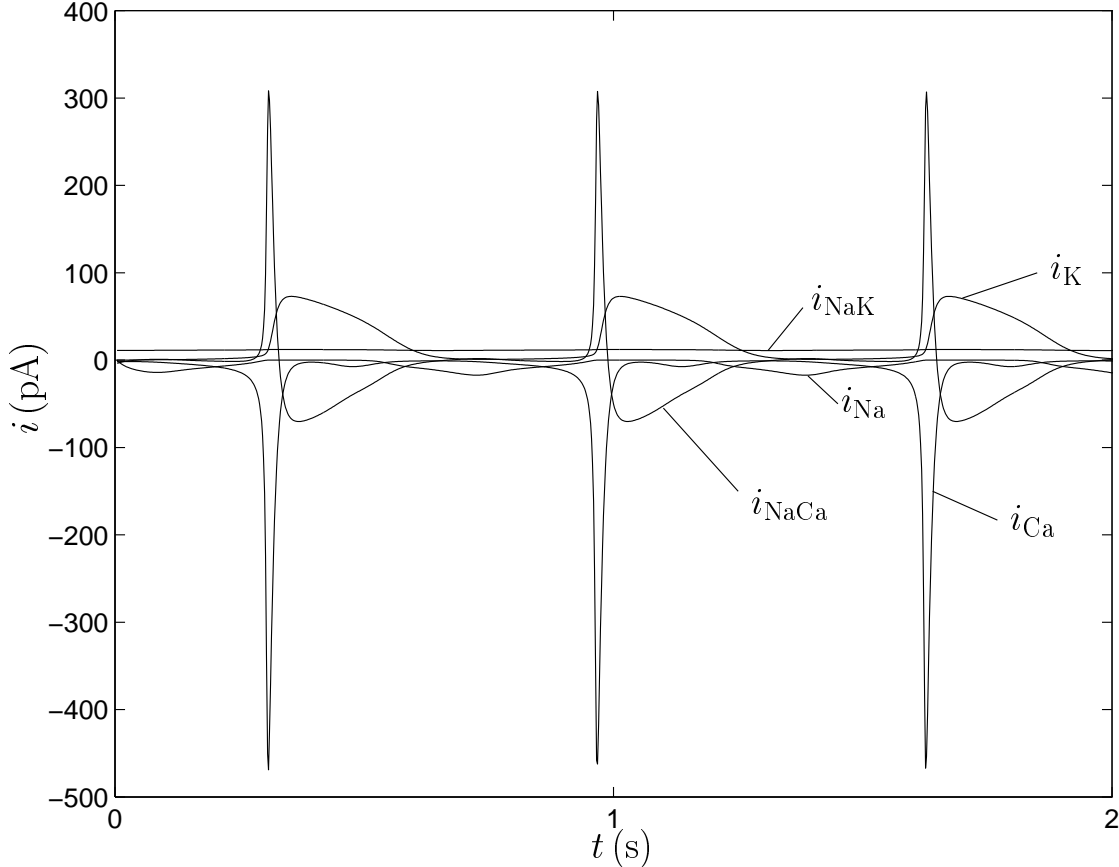


FIG. 2. Membrane currents in a simple ionic model of a rabbit sinoatrial node cell. We show the outward delayed rectifying potassium current ( $i_K$ ), the inward calcium current ( $i_{Ca}$ ), the inward sodium current ( $i_{Na}$ ), the sodium calcium exchange current ( $i_{NaCa}$ ) and the sodium potassium pump current ( $i_{NaK}$ ). These computations used the initial conditions in table IV.

The results are presented in Fig. 4. After approximately 1500 seconds (25 minutes) the system appears to reach a stable fixed point with the following intracellular ionic concentrations:

$$\begin{aligned} [K]_i &= 115.842881 \text{ mM} , \\ [Ca]_i &= 4.485016 \cdot 10^{-5} \text{ mM} , \\ [Na]_i &= 33.548671 \text{ mM} . \end{aligned} \tag{67}$$

At 2500 seconds we kick the cell with a 20 pA pulse of potassium ions with duration 50 ms to see if it wants to oscillate. It surely does, and the oscillations soon become almost identical to the original oscillations present in Fig. 2 and 3 (you can not see this from Fig. 4 since the time scale is much too big). The values for the concentrations only differ

slightly from the initial conditions in table IV, as indicated by the final concentrations calculated by the program (at 5000 seconds):

$$\begin{aligned} [K]_i &= 131.075490 \text{ mM}, \\ [Ca]_i &= 6.827191 \cdot 10^{-4} \text{ mM}, \\ [Na]_i &= 18.320693 \text{ mM}. \end{aligned} \tag{68}$$

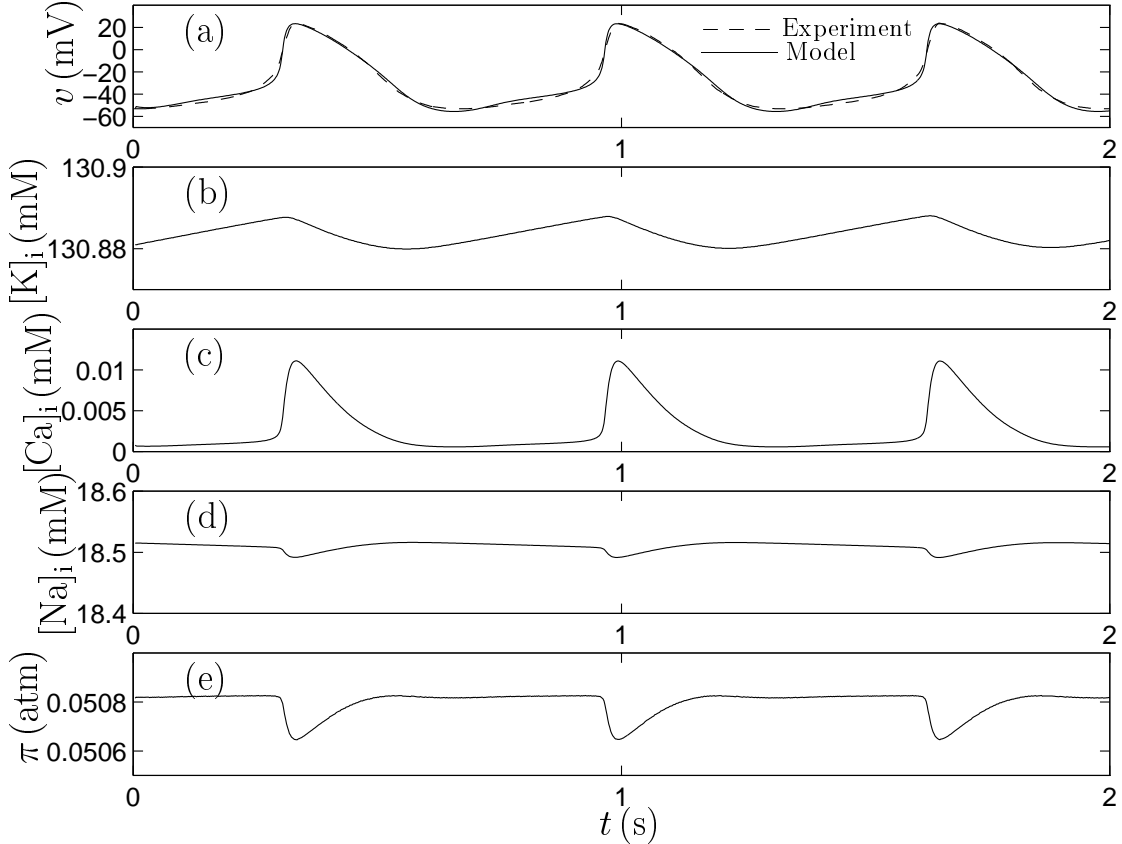


FIG. 3. Membrane potential, intracellular ionic concentrations and osmotic pressure of a rabbit sinoatrial node cell. (a) Model-generated (solid line) and experimentally recorded (dashed line) action potential waveform, (b) potassium concentration  $[K]_i$ , (c) calcium concentration  $[Ca]_i$ , (d) sodium concentration  $[Na]_i$  and (e) the osmotic pressure  $\pi$  across the cell membrane. These computations used the initial conditions in table IV.

The trajectory reaches the limit cycle at 2560 seconds, and there is no drift in the intracellular ionic concentrations from this point to the end of the simulation (at 5000 seconds). The long time simulation in Fig. 4 is a numerical indication that the oscillation in Fig. 2 and 3 is indeed a stable limit cycle. However, the model also has a stable fixed point given by (67).

## V. DISCUSSION

We have presented a simple model for the cells of the rabbit sinoatrial node. Our model involves only  $Na^+$ ,  $K^+$ , and  $Ca^{2+}$  ions, their respective channels, the  $Na^+$ ,  $Ca^{2+}$  exchanger, and the  $Na^+$ ,  $K^+$  pump. The equations were derived using basic physical principles and conservation laws. Since the only source of energy in our model is the sodium pump, we could easily track the flow of energy in our system. We showed that the pump works to generate a transmembrane voltage, pressure gradient and ionic concentration gradients. Our equations also accounted for the energy lost due to downhill ionic fluxes through the exchanger and channels. A prediction of osmotic pressure variations was a novel result of our energy analysis.

The intracellular ionic concentrations are dynamic variables in our model, governed by the conservation equations (41), (42), and (43). This allowed us to replace the standard differential equation for the voltage (40) with the algebraic equation (51). Although a number of other ionic models also keep track of intracellular ionic concentrations (see Wilders (1993)), we are unaware of any other model using an algebraic equation for the membrane potential. Models that use the standard voltage differential equation (40) have a superfluous extra dimension. Furthermore, the initial conditions for this extra differential equation cannot be chosen independently of the initial conditions of the conservation equations (41), (42), and (43) – otherwise, the computed membrane potential will be erroneous. For these reasons, we suggest that our algebraic expression for the membrane potential should replace the standard voltage differential equation in models where intracellular ionic concentrations are dynamic variables.

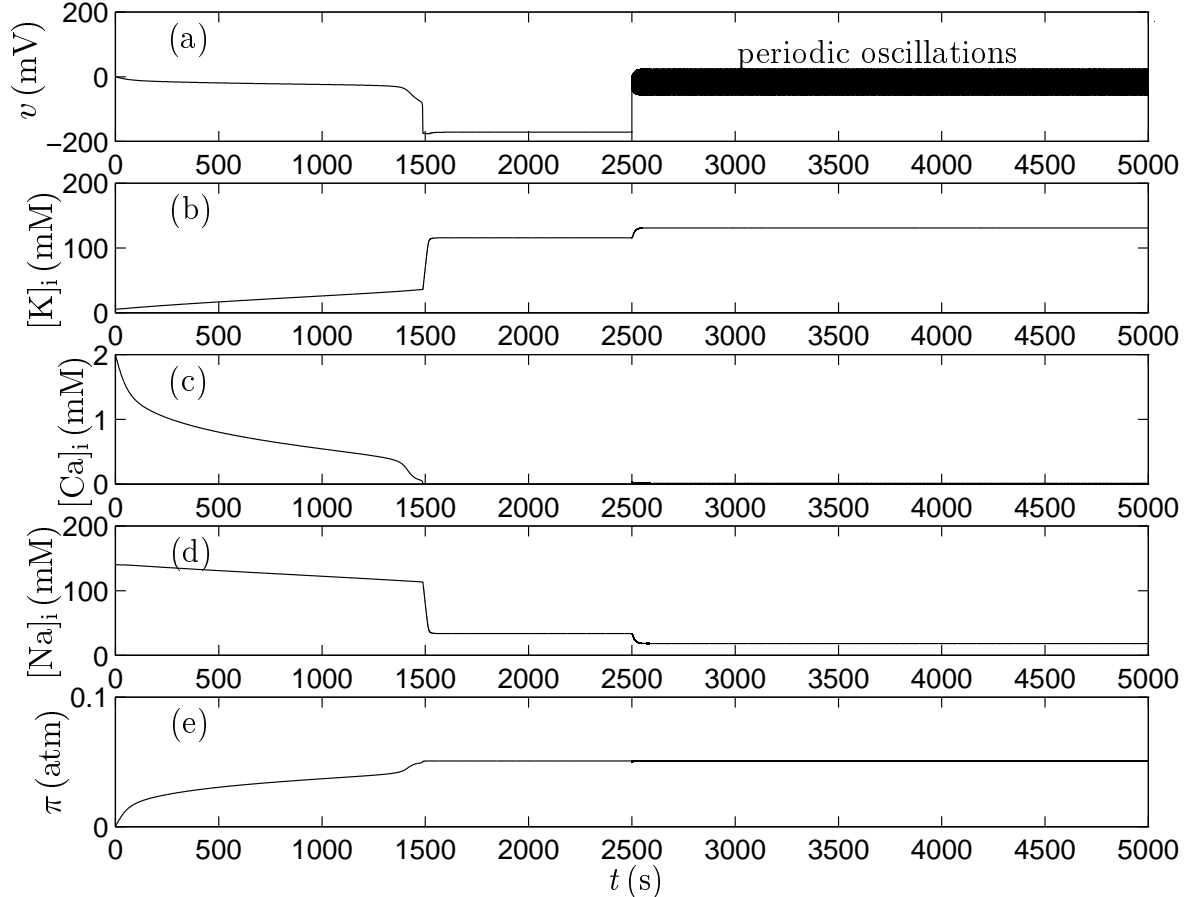


FIG. 4. Long time simulation showing the membrane potential, intracellular ionic concentrations and osmotic pressure starting with equal intracellular and extracellular concentrations:  $[K]_i = 5.4$  mM,  $[Ca]_i = 2$  mM and  $[Na]_i = 140$  mM. The cell is given a 20 pA pulse of potassium with duration 50 ms after 2500 seconds of the simulation. (a) Membrane potential  $v$ , (b) potassium concentration  $[K]_i$ , (c) calcium concentration  $[Ca]_i$ , (d) sodium concentration  $[Na]_i$  and (e) the osmotic pressure  $\pi$  across the cell membrane.

Our model does not include funny current ( $i_f$ ), ATP sensitive channels, stretch-activated channels, or other ion channels that may be important (Boyett, 1996). We also ignored the effect of calcium uptake and release from the sarcoplasmic reticulum, and the effect of anions like chloride. We have assumed that the ionic channels are governed by a Markov process. However, since transmembrane proteins can have a large number of conformational states (Liebovitch, 1995), perhaps a fractal model for the gating would be a better approach. We assumed that the maximum of the activation/inactivation time constant occurs at the same voltage as the inflection point of the sigmoidal steady state activation/inactivation curve. Also, we have assumed that the cell volume is constant. While such assumptions reduce the number of parameters in the model, they may also result in discrepancies with experiment. A natural extension of our model would include a variable cell volume.

Finally, we would like to point out that our model is based on experiments where some were conducted at room temperature (22–24°C) (Baruscotti *et al.*, 1996; Muramatsu *et al.*, 1996), while others were performed at 37°C

(Shibasaki, 1987; Hagiwara *et al.*, 1988; Sakai *et al.*, 1996). It is not clear what affect this inconsistency had on the accuracy of our model.

The values of the parameters  $g_{Ca}$ ,  $g_{Na}$ ,  $g_K$ ,  $k_{NaK}$  and  $k_{NaCa}$ , given in table III, are only an estimate of the actual physiological parameters. We did not systematically study the dynamics of the model for different parameters. However, interested readers can download the source code for the model (in both UNIX and Windows NT environments) from the following internet site:

<http://www.physio.mcgill.ca/guevaralab/singlecell.htm>

We hope that future experiments will help us to discriminate between the different parameter sets that reproduce the experimentally recorded action potentials.

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